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Alpha-SMA expression in hepatic stellate cells and quantitative analysis of hepatic fibrosis in cirrhosis and in recurrent chronic hepatitis after liver transplantation

G. Carpino [a], S. Morini [b], S. Ginanni Corradini [c], A. Franchitto [a], M. Merli [c], M. Siciliano [c], F. Gentili [c], A. Onetti Muda [d], P. Berloco [e], M. Rossi [e], A.F Attili [c], E. Gaudio [a, b, *]

[a] *Department of Human Anatomy, University of Rome 'La Sapienza', via Alfonso Borelli, 50, 00161 Rome, Italy*
[b] *Department of Biomedical Research, University "Campus Bio-Medico" of Rome, Italy*
[c] *Department of Clinical Medicine, II Division of Gastroenterology, University of Rome "La Sapienza", Italy*
[d] *Department of Experimental Medicine and Pathology, University of Rome "La Sapienza", Italy*
[e] *Department of Surgery and Organs Transplantation, University of Rome "La Sapienza", Italy*

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Abstract

Background. The alpha isotype of actin expressed by hepatic stellate cells reflects their activation to myofibroblast-like cell and has been directly related to experimental liver fibrogenesis, and indirectly to human fibrosis in chronic liver disease.

Aims. To evaluate the changes in distribution and percentage of alpha-smooth muscle actin-positive hepatic stellate cells and the correlation with the degree of the fibrosis in cirrhotic livers, as well as in patients with recurrent HCV chronic hepatitis after liver transplantation.

Methods. Human liver biopsies were divided in four groups: (1) normal livers obtained from cadaveric liver donors ($n = 35$), (2) cirrhosis post-HBV hepatitis ($n = 11$), (3) cirrhosis post-HCV hepatitis ($n = 10$), and (4) post-transplant recurrent HCV chronic hepatitis ($n = 13$). Samples were stained with anti-alpha-smooth muscle actin antibody by immunoperoxidase method and semi-quantitatively evaluated. Liver fibrosis was assessed from specimens stained with Masson's trichrome and quantified by computer image analysis.

Results. The percentage of alpha-smooth muscle actin-positive hepatic stellate cells was significantly higher in the HBV cirrhosis, HCV cirrhosis and post-transplant HCV recurrent hepatitis groups (36.1 ± 15.2, 23.8 ± 19.7 and $27.8 \pm 16.4\%$, respectively) compared to the liver donor group ($2.9 \pm 4.0\%$). The alpha-smooth muscle actin-positive hepatic stellate cells to fibrous tissue ratio were significantly higher in the post-transplant recurrent HCV hepatitis group (2.36 ± 1.12) compared to both the donor livers and the HCV cirrhosis groups (0.74 ± 1.09 and 1.03 ± 0.91, respectively). The alpha-smooth muscle actin-positive hepatic stellate cell percentage and fibrosis correlated positively in the post-transplant recurrent HCV hepatitis group and negatively in the HCV cirrhosis group. No difference in the immunohistochemical and morphometrical variables was found between the HCV cirrhosis and HBV cirrhosis groups.

Conclusions. These results indirectly confirm that, in vivo, alpha-smooth muscle actin expression is a reliable marker of hepatic stellate cells activation which precedes fibrous tissue deposition even in the setting of recurrent HCV chronic hepatitis after liver transplantation, and it could be useful to identify the earliest stages of hepatic fibrosis and monitoring the efficacy of the therapy. In the presence of advanced cirrhosis other factors, rather than alpha-smooth muscle actin-positive hepatic stellate cells, may sustain fibrosis deposition.

Keywords: Hepatic stellate cells; Liver cirrhosis; Liver fibrosis; Post-transplant recurrent HCV hepatitis

1. Introduction

Hepatic fibrosis, the common final manifestation of several chronic liver diseases, is the result of a prominent accumulation of extracellular matrix (ECM) materials

* Corresponding author. Tel.: +39 06 49 91 80 62; fax: +39 06 49 91 80 62.
E-mail address: eugenio.gaudio@uniromal.it (E. Gaudio).

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and ultimately can lead to cirrhosis. Several studies have described that hepatic stellate cells (HSC) play a central role in the pathogenesis of fibrosis [1–5].

In normal liver, HSCs are non-parenchymal, quiescent cells that have three main physiological functions as Vitamin A storage, production of ECM in the space of Disse and a role in the regulation of the sinusoidal microcirculatory flow [4,6]. However, numerous studies indicate that in response to liver injury HSCs undergo an 'activation' process characterised by proliferation and myofibroblastic transformation [1,3,5]. Activated HSCs increase production of fibrillar collagen (collagen type-I and type-III) and they are the major cell type for matrix production in damaged liver tissue [5]. In response to their activation HSCs show an intense cytoplasmic alpha-smooth muscle actin (alpha-SMA) immunoreactivity. Alpha-SMA is an actin isoform and a specific marker for smooth muscle cell differentiation [7]. Therefore, alpha-SMA expression has been used to identify activated HSCs that show a myofibroblastic phenotype [3,8,9].

The in vivo evidence that alpha-SMA-positive HSCs are responsible for fibrotic tissue accumulation in human chronic liver disease, can be indirectly based on their increased number in the liver of patients with chronic liver disease, compared to the normal liver, and on the correlation between their number with the extent of fibrosis. Discrepant data, some obtained on a very small number of samples, have been reported on the number or percentage of anti-alpha-SMA-positive HSCs in normal liver [8–16]. On the other hand, the number of alpha-SMA-positive HSCs has been related to the extent of human hepatic fibrosis assayed by quantitative morphometrical analysis, in only one study performed in patients with chronic hepatitis C in which no normal liver was examined [17].

HCV recurrent hepatitis is a common clinical problem after liver transplantation [18]. However, only one study has measured the number of alpha-SMA-positive HSCs in HCV recurrent, mostly acute, hepatitis which was found to predict the subsequent evolution to cirrhosis, while no data on the relation between the number of alpha-SMA-positive HSCs and fibrosis quantification in HCV recurrent chronic hepatitis have been reported [14].

In this study, we measured the percentage of alpha-SMA-positive HSCs and quantified the volume fraction occupied by fibrosis using morphometry in the liver of HCV and HBV cirrhotic patients undergoing liver transplantation, in allografted livers from patients with recurrent chronic HCV hepatitis and in a large number of normal livers. Our aims were: (i) to evaluate the correlation between alpha-SMA-positive HSCs and the degree of fibrosis at different stages of chronic liver disease and clarify whether alpha-SMA expression is a precocious marker of fibrosis even in the post-transplant setting; (ii) to determinate possible differences of alpha-SMA expression related with the aetiology of the disease.

2. Materials and methods

This study involved 66 hepatic tissue samples divided in donor livers (DL; $n = 35$), HBV cirrhosis (HBV-C; $n = 11$), HCV cirrhosis (HBV-C; $n = 10$) and post-transplant HCV recurrent hepatitis (HCV-PTR; $n = 13$) groups. Liver specimens were obtained from heart-beating cadaveric liver donors at laparatomy for the DL group, from explanted cirrhotic liver at liver transplantation for the HBV-C and HCV-C groups, and from percutaneous needle biopsies with histological aspect of recurrent HCV hepatitis and fibrosis after orthotopic liver transplantation for the HCV-PTR group, taken for diagnostic purpose with the informed consent of the patients. The study followed the ethical procedures approved for the Policlinico Umberto I of the University of Rome "La Sapienza".

2.1. Light microscopy and immunohistochemistry

Liver fragments were fixed in buffered formalin for 2–4 h and embedded in paraffin with melting point 55–57 °C. Three to four micrometers sections were cut and stained with haematoxylin-eosin and Masson's trichrome stains.

For immunohistochemical studies, the sections were mounted on glass slides coated with 0.1% poly-L-lysine. After deparaffination, and subsequent blockage of the endogenous peroxidase activity by incubation in 2.5% methanolic hydrogen peroxide (30 min), the endogenous biotin was blocked by Biotin Blocking System (Dako, Milan, Italy) according to the instructions received from the vendor. The sections were then washed three times in phosphate-buffered saline (PBS). Mouse monoclonal anti-alpha-SMA antibody (Dako 1A4) diluted 1:40 was used as primary antibody. The sections were incubated for 1 h at room temperature. After three washings in PBS, the sections were incubated for 30 min with the appropriate secondary biotinylated antibody labelled with the Avidin-Biotin Complex (LSAB, Dako code K0675). The sections were developed with 3-3′ diaminobenzidine and finally counterstained with haematoxylin. Negative controls were performed using normal mouse antiserum instead of the primary antibody, which uniformly demonstrated no reaction.

Estimation of the number of anti-alpha-SMA immunoreactive HSCs was done independently by two researchers. Intra-observer agreement resulted higher than 90%. HSCs were distinguished from the other myofibroblasts of the liver (such as portal myofibroblasts, interface myofibroblasts and septal myofibroblasts) by their specific position in the liver parenchyma [19,20]. The number of alpha-SMA-positive HSCs and alpha-SMA-negative HSCs was counted under light microscope at 200 magnifications: only the cells which displayed nuclei on the section were considered. For each slide, at least 7–10 microscopic fields were randomly chosen. The percentage of alpha-SMA-positive HSCs was calculated on the total number of HSCs counted in each slide. The average percentage of activated HSCs was then calculated for each group.

In the HCV-PTR group, histopathological lesions of recurrent chronic HCV hepatitis were evaluated according to

Table 1
Demographics and serum aminotransferases of study populations

	DL ($n=35$)	HBV-C ($n=11$)	HCV-C ($n=10$	HCV-PTR ($n=13$)
Age (years)	49.6 ± 19.3	55.9 ± 5.8	57.1 ± 4.9	55.5 ± 5.7
Male sex (n) (%)	12 (34)	10 (91)[a]	6 (60)	8 (62)
AST (U/l)	45.0 ± 26.5[b]	65.8 ± 20.1[c]	70.4 ± 29.0	131.9 ± 98.5
ALT (U/l)	38.5 ± 29.3[d,e]	52.6 ± 14.1	55.0 ± 23.0	134.2 ± 120.0[f]

[a] $P < .01$ by χ^2 test.
[b] $P < .01$ vs. HBV-C, HCV-C and HCV-PTR.
[c] $P < .05$ vs. HCV-PTR.
[d] $P < .001$ vs. HCV-PTR.
[e] $P < .05$ vs. HBV-C and HCV-C.
[f] $P < .05$ vs. HCV-C and HBV-C.

the histology activity index (HAI) of Knodell et al. [21]; however, necro-inflammatory and fibrosis scores were given separately, to distinguish ongoing hepatitis from parenchymal remodelling with fibrosis. In the HBV-C and HCV-C groups, active cirrhosis was defined by the presence of fibrotic septa heavily infiltrated by inflammatory cells, the latter also invading the nearby parenchyma; the lack of the heavy inflammatory infiltrate and, more importantly, of interface activity defined inactive cirrhosis.

2.2. Morphometrical analysis

Quantitative analysis was performed on specimens stained with Masson's trichrome as previously described [22,23]. In brief, light microscopy micropictures were captured by a Videocam (SPOT Insight; Diagnostic Instrument, Inc., Sterling Heights, MI) connected to an Olympus BX-51 light microscopy (Olympus, Tokyo, Japan) and processed with an Image Analysis System (Delta Sistemi, Roma, Italy). The green-stained collagen fibres were automatically measured as the volume fraction of the entire liver tissue specimen, including the collagen fibres that normally existed in the portal tract or central vein. The volume fraction occupied respectively by portal triads (arteries, veins and bile ducts) and by the parenchyma (hepatocytes and sinusoids) was then separately measured.

2.3. Statistical analysis

Results are presented as the mean ± S.D. Statistical analysis was conducted by using the Mann–Whitney-U test. To assess significant correlations, the Pearson or Spearman correlation coefficients were calculated in case data had normal or not-normal distribution, respectively. Values $P < .05$ were regarded as statistically significant.

3. Results

3.1. Study population characteristics

The demographics and serum aminotransferase concentrations of the DL, HBV-C, HCV-C and HCV-PTR groups are shown in Table 1. The percentage of males was significantly higher in the HBV-C group. The HCV-PTR group had higher serum aminotansferase values than all the other groups. The HBV-C and HCV-C groups did not differ in terms of MELD score (13.7 ± 4.2 and 11.8 ± 6.7, respectively).

3.2. Hepatic histology

The diagnosis of cirrhosis was confirmed by histological examination of the explanted liver in all cases of the HBV-C and HCV-C groups. The biopsies collected in the HCV-PTR group were performed when clinically indicated at a mean distance of 18.3 ± 20.2 months after the transplantation. The biopsies of the HCV-PTR group showed chronic hepatitis in all cases with low grading (6.2 ± 3.4) and staging (1.8 ± 1.0) Knodell scores.

3.3. Hepatic alpha-SMA HSC immunohistochemistry

Fig. 1 shows the box plots of the percentage of alpha-SMA-positive HSCs that was found in the four groups. The percentage of alpha-SMA-positive HSCs was signifi-

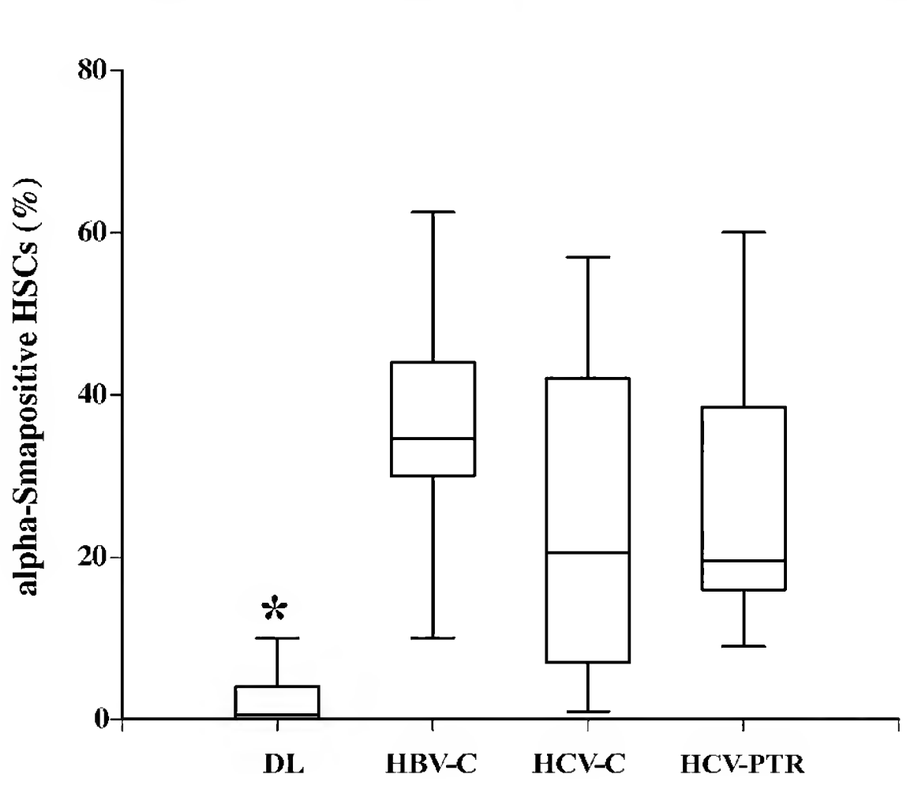

Fig. 1. Box plots of the percentage of alpha-SMA-positive HSCs in hepatic tissue of cirrhotic patients, normal DLs and patients with recurrent HCV hepatitis after liver transplantation. DL, donor livers; HBV-C, HBV cirrhosis; HCV-C, HCV cirrhosis; HCV-PTR, post-transplant HCV recurrent hepatitis. *$P < .001$ vs. all the other groups.

cantly higher in the HBV-C, HCV-C and HCV-PTR groups (36.1 ± 15.2, 23.8 ± 19.7 and 26.8 ± 14.6%, respectively) compared to the DL group (2.9 ± 4.0%). No other inter-group difference was found in the percentage of alpha-SMA-positive HSCs.

With regard to the distribution of alpha-SMA-positive HSCs, in the DL group very few alpha-SMA-positive HSCs were found only along the sinusoids, mostly in the peripheral zones of the hepatic lobule close to the portal spaces (Fig. 2a).

In the HBV-C and HCV-C groups, alpha-SMA-positive HSCs were strongly and diffusely immunostained (Fig. 2b). In active cirrhosis, many HSCs were present in the expanding septa as well as in the perisinusoidal spaces of residual hepatic parenchyma (Fig. 2c), but in areas demonstrating steatosis and/or regenerative activity alpha-SMA-positive HSCs were confined to the periphery of the regenerative plates. HSCs varied in shape and size, although most of them stretched long cytoplasmic processes along the endothelial lining (Fig. 2d).

In the HCV-PTR group, the stronger alpha-SMA expression was detected at the site of piecemeal necrosis.

3.4. Hepatic morphometry

The volume fraction of fibrosis and that occupied by arteries and veins, bile ducts, hepatocytes and sinusoids is reported in Table 2.

In the DL group, the volume fraction of fibrosis was represented only by the connective tissue of the portal tracts. The volume fraction of fibrosis was significantly higher in the HBV-C, HCV-C and HCV-PTR groups compared to the DL group and in the HBV-C and HCV-C groups compared to the HCV-PTR group. The volume fraction of fibrosis did not differ between the HBV-C and the HCV-C group.

The volume fraction occupied by arteries and veins and by bile ducts was higher in the HBV-C, HCV-C and HCV-PTR groups compared to the DL group. The volume fraction occupied by arteries and veins and by bile ducts did not differ between the HBV-C and the HCV-C group.

The volume fraction occupied by hepatocytes and by sinusoids was significantly lower in the HBV-C, HCV-C and HCV-PTR groups compared to the DL group, and in the HBV-C and HCV-C groups compared to the HCV-PTR group. The

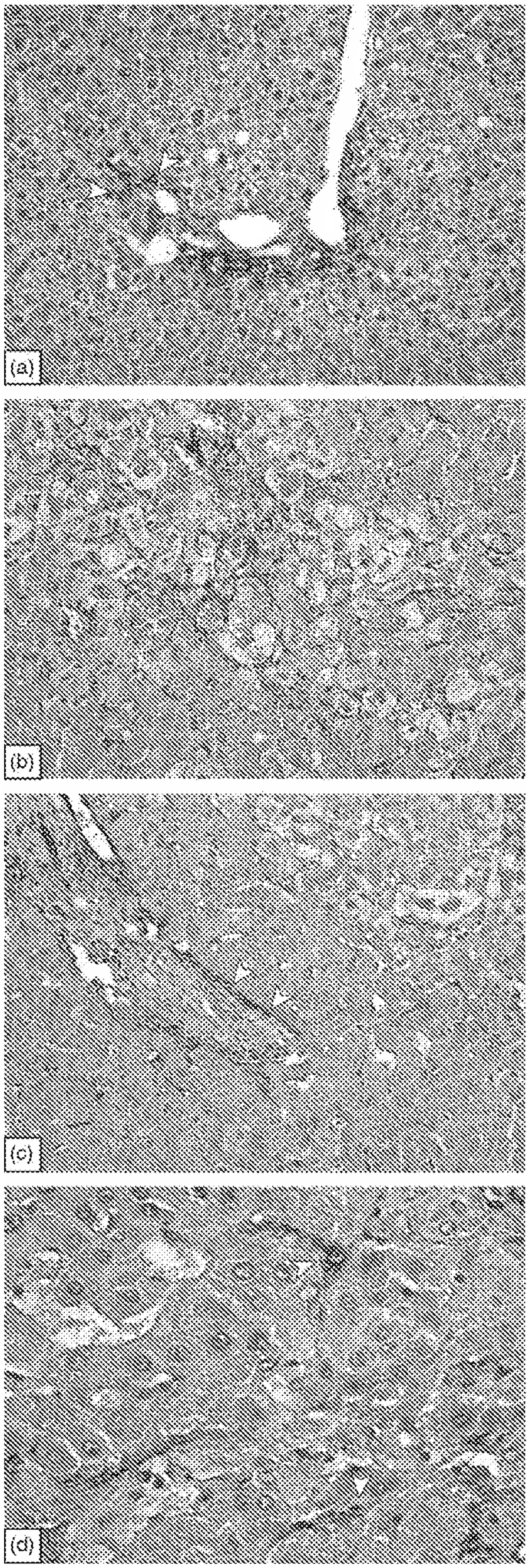

Fig. 2. (a) Alpha-SMA-positive HSCs, in DL. Very few alpha-SMA-positive HSCs are present only along the sinusoids (arrowheads), in the peripheral zones of the hepatic lobule close to the portal spaces. Immunohistochemistry for alpha-SMA, original magnification 10×. (b) Alpha-SMA-positive HSCs in HCV-C. Activated HSCs are strongly and diffusely brown-stained inside the liver. Immunohistochemistry for alpha-SMA, original magnification 20×. (c) Alpha-SMA-positive HSCs in active cirrhosis. Many HSCs are visible in expanding septa at the interface with the hepatocytes (arrowheads), as well as in the perisinusoidal spaces of residual hepatic parenchyma. Immunohistochemistry for alpha-SMA, original magnification 20×. (d) Detail of alpha-SMA-positive HSCs. Activated HSCs (arrowheads) show larger size and long cytoplasmic processes expanding along both the hepatocytes and the endothelial cell lining. Immunohistochemistry for alpha-SMA, original magnification 40×.

Table 2
Distribution of parenchymal and non-parenchimal compartments at hepatic morphometrical analysis

	Fibrous tissue	Arteries and veins	Bile ducts	Hepatocytes	Sinusoids
DL	4.13 ± 0.78	1.97 ± 0.31	0.21 ± 0.07	76.18 ± 2.74	17.52 ± 2.90
HBV-C	23.63 ± 3.82[a]	9.12 ± 1.38[a]	2.58 ± 0.37[a]	52.95 ± 4.87[a]	11.71 ± 0.48[a]
HCV-C	25.68 ± 4.14[a]	8.89 ± 1.40[a]	2.35 ± 0.22[a]	51.07 ± 4.17[a]	12.02 ± 1.94[a]
HCV-PTR	13.62 ± 8.23[a,b]	2.73 ± 1.00[c,d]	0.35 ± 0.19[c,d]	68.76 ± 7.55[a,c]	14.14 ± 1.80[b,d]

[a] $P < .0001$ vs. DL.
[b] $P < .02$ vs. HBV-C and HCV-C.
[c] $P < .001$ vs. HBV-C and HCV-C.
[d] $P < .01$ vs. DL.

volume fraction occupied by hepatocytes and by sinusoids did not differ between the HBV-C and the HCV-C group.

3.5. Relationships among immunohistochemical, morphometrical and histological parameters

Fig. 3 shows the box plots of the ratio between the percentage of alpha-SMA-positive HSCs and the volume fraction of fibrosis detected at morphometrical analysis in the four groups. The alpha-SMA-positive HSCs to fibrous tissue ratio was significantly higher in the HBV-C and HCV-PTR groups (1.57 ± 0.72 and 2.50 ± 1.17, respectively) compared to the DL group (0.74 ± 1.09). The alpha-SMA-positive HSCs to fibrous tissue ratio was significantly lower in the HCV-C group (1.03 ± 0.91) compared to the HCV-PTR group. No other intergroup difference was found in the alpha-SMA-positive HSCs to fibrous tissue ratio. A positive correlation was found between the alpha-SMA-positive HSC percentage and the volume fraction of fibrosis in liver tissue measured by morphometrical analysis in the HCV-PTR group (Fig. 4, panel A). At variance, as shown in Fig. 4 panel B, a negative correlation was found between the alpha-SMA-positive HSC percentage and the volume fraction of fibrosis in liver tissue when only the HCV-C group was considered. No correlation between the alpha-SMA-positive HSC percentage and the volume fraction of fibrosis in liver tissue was found in the HBV-C group (Fig. 4, panel C) and in the DL group (data not shown).

A positive correlation was also present when the alpha-SMA-positive HSC percentage was plotted against the histological fibrosis score ($y = 12.863x + 5.0125$; Spearman $r = 0.807$; $P < .01$; data not shown).

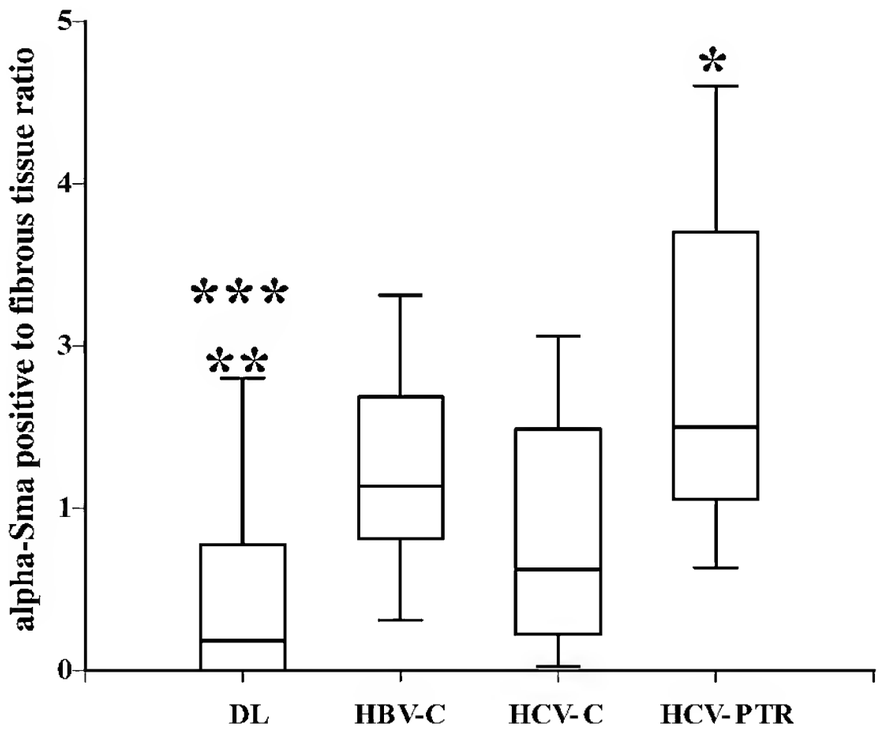

Fig. 3. Box plots of the ratio between the percentage of alpha-SMA-positive HSCs and the volume fraction of fibrosis in hepatic tissue of cirrhotic patients, normal DLs and patients with recurrent HCV hepatitis after liver transplantation. DL, donor livers; HBV-C, HBV cirrhosis; HCV-C, HCV cirrhosis; HCV-PTR, post-transplant HCV recurrent hepatitis. $^{*}P < .02$ vs. HCV-C, $^{**}P < .005$ vs. HBV-C, $^{***}P < .0005$ vs. HCV-PTR.

4. Discussion

In the present study, we provide indirect 'in vivo' evidences that HSC activation is a key factor in the natural history of human HCV and HBV chronic liver disease and that it precedes fibrotic hepatic accumulation. The first evidence is that the percentage of alpha-SMA HSCs was 9.7, 8.3 and 12.6 folds higher in the HCV-PTR, HCV-C and HBV-C groups, respectively, when compared to the DL group. Our findings of the increase of activated HSCs in chronic liver disease compared to normal livers are in agreement with previous reports obtained in both non-transplanted patients and in recurrent, mostly acute, post-transplantation HCV hepatitis [8,10–17]. The magnitude of the increase of alpha-SMA HSC expression by immunohistochemistry in chronic liver disease with respect to normal livers depends on the wide differences in number and distribution of positive HSCs detected by different investigators in normal livers [8,10,11,13–16], probably due to variations in assay sensitivity among laboratories and in the number of normal liver samples studied. In the present study, we found that 3% of HSCs were alpha-SMA-positive in a large number of normal livers.

The second evidence of the present study that relates HSC activation to fibrosis was that the volume fraction of fibrosis in liver tissue was 3.3, 6.2 and 5.7 folds higher in the HCV-PTR, in the HCV-C and in HBV-C groups, respectively, when compared to the DL group. As a consequence, the alpha-SMA-positive HSC to fibrosis ratio in the HCV-PTR group was 2.3 and 1.5 folds higher than in the HCV-C and HBV-C groups, respectively, suggesting that even in the settings of human post-transplantation HCV re-infection, HSC activation preceded fibrotic tissue accumulation. Unfortunately, our data on recurrent hepatitis were referred only to post-transplant HCV group; in fact, post-transplant HBV-recurrent hepatitis is very

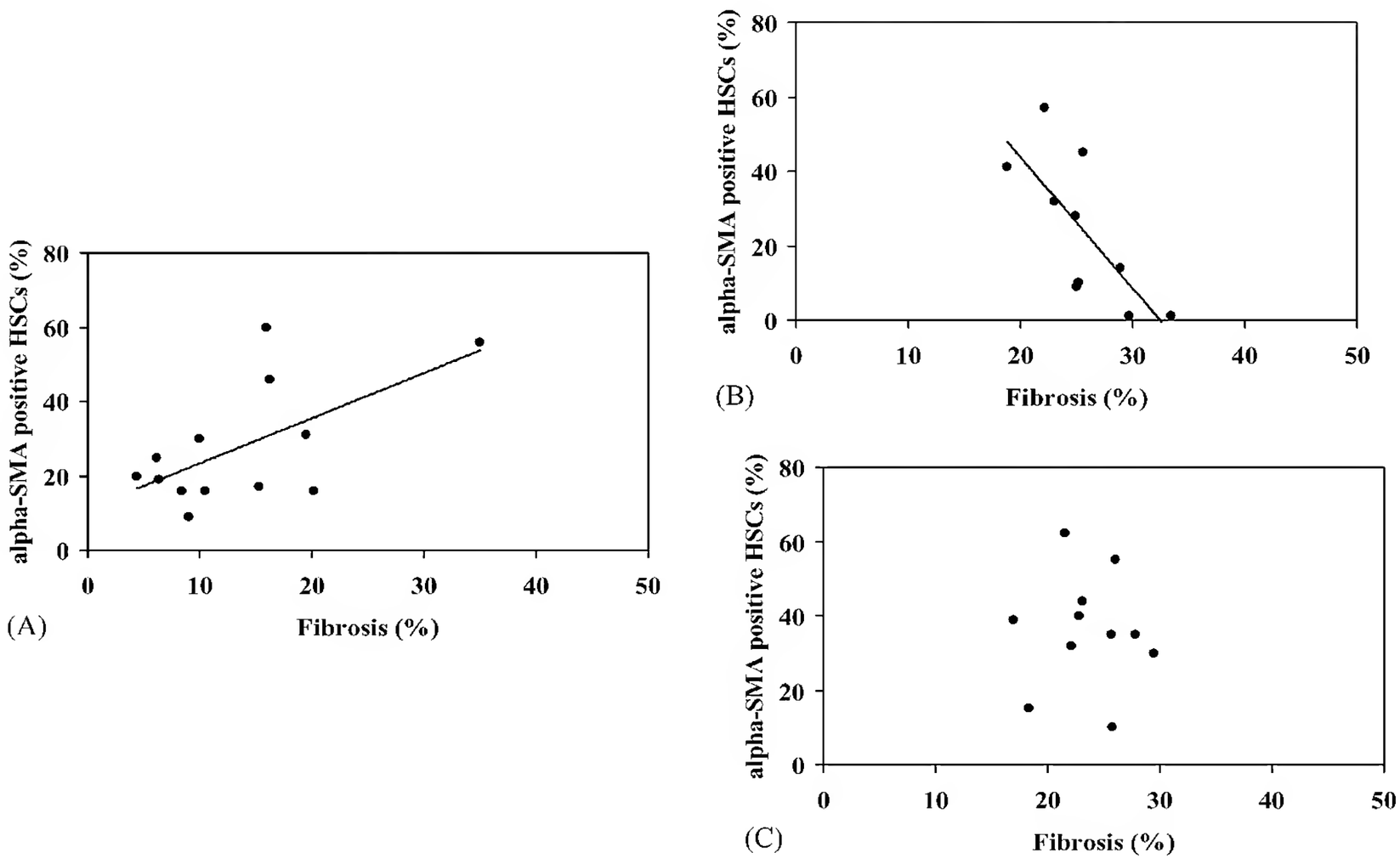

Fig. 4. Panel A: positive correlation ($y = 3219x + 10.2$; Pearson $r = 0.828$; $P < .005$) between the percentages of alpha-SMA-positive HSCs and the volume fraction of fibrosis in liver tissue in the HCV-PTR group. Panel B: negative correlation ($y = -3.5311x + 114.46$; Pearson $r = -0.744$; $P < .02$) between the percentages of alpha-SMA-positive HSCs and the volume fraction of fibrosis in liver tissue in the HCV-C group. Panel C: no correlation (Pearson $r = -0.024$) between the percentages of alpha-SMA-positive HSCs and the volume fraction of fibrosis in liver tissue in the HBV-C group.

uncommon with the effective antiviral prophilaxis available nowadays. In addition, in the present study no biopsies of both HBV and HCV chronic hepatitis non-transplanted patients were available. On the other hand, post-transplantation HCV re-infection represents a human model of the earlier stage of fibrosis [14,17], which is very useful because we exactly know the moment of the infection. Our results are in keeping with a previous study performed in the liver transplantation settings by Guido et al. showing that the number of alpha-SMA-positive HSCs in HCV recurrent, mostly acute, hepatitis 3–6 months after transplantation was related to a subsequent cirrhotic evolution [14].

A further line of evidence of the present study that relates HSC activation to fibrosis was the positive correlation between the alpha-SMA-positive HSC percentage and the extent of fibrosis measured by both morphometrical analysis and histological scoring in the HCV-PTR group. Variations in assay sensitivity among laboratories could explain the lack of association between the prevalence of alpha-SMA-positive HSCs and fibrosis severity in chronic liver disease in non-transplanted patients reported by Levy et al., while in their paper only a subpopulation of fibroblast activation protein-positive HSCs correlated with fibrosis [15]. On the other hand, two previous studies on chronic liver disease in non-transplanted patients are in agreement with our finding of a positive correlation between the alpha-SMA-positive HSC percentage and the extent of fibrosis [16,17]. Our data agree with the models of fibrogenesis proposed by several authors [24,25] where the activation of HSC are the central events in hepatic fibrosis. The differentiation of HSCs precedes the production of connective tissue and the presence of HSCs expressing a myofibroblast-like phenotype is necessary for deposition of scar molecules [5]. In addition, appearance of the alpha-SMA HSCs as observed in the HCV-PTR group before the massive accumulation of scar molecules could be considered as an useful marker for an early diagnosis of post-transplant hepatic diseases that will result in severe fibrosis [14]. As a consequence, alpha-SMA expression could have a prognostic role also in the follow-up after orthotopic liver transplantation.

The identification of activated HSCs as a major source of ECM in fibrotic livers [26,27] leads to considering them as a major target for the treatment [5,28,29]. In addition, alpha-SMA expression could be useful not only as an early marker of hepatic fibrogenesis necessary to begin a treatment before the process become irreversible, but also as a marker for monitoring the efficacy of the therapy [12,17].

At variance from the HCV-PTR group, in the HCV-C group we found a negative correlation between the alpha-SMA-positive HSC percentage and the volume fraction of fibrosis detected at morphometry, while we failed to find any correlation in the HBV-C group. The lack of a positive correlation between the alpha-SMA-positive HSC percentage and the volume fraction of fibrosis in the cirrhotic groups suggests that, once advanced cirrhosis is established and progresses, other HSCs phenotypes and/or other cells (such as portal, septal and perivenular fibroblasts) support fibrogenesis in addition to alpha-SMA-positive HSCs [14]. Moreover, the evidence that HCV proteins can directly induce fibrogenic effects in HSCs [30] and the decreased

intra-hepatic HCV replication in advanced cirrhosis [31] could explain the negative correlation between the alpha-SMA-positive HSC percentage and the volume fraction of fibrosis in the HCV-C group found in the present study.

By contrast, the lack of any correlation in the HBV-C group between the alpha-SMA-positive HSC percentage and the volume fraction of fibrosis could be also explained because HBV cirrhotic patients are currently submitted to liver transplantation when very low or undetectable HBV serum levels have been obtained, either spontaneously or under antiviral treatment. The latter hypothesis is in agreement with the positive correlation observed between the intra-hepatic HBV content and degree of fibrosis [32]. Moreover, Kweon et al. [33] showed that lamivudine treatment in HBV chronic hepatitis decreased HSCs activation even in the cases where the post-treatment biopsy demonstrated unchanged or worsened fibrosis score.

Deeper studies should explain these data, and other markers might be useful. At present, alpha-SMA expression is considered as a reliable marker of the activated HSCs [3,8,11], while other markers seem to present problems not yet solved. In fact, GFAP is expressed only in a sub-population of activated HSC; N-CAM is expressed also in quiescent HSCs [4]; FAP, considered as a good marker of the production of collagens, appears mainly in the advanced stages of fibrogenesis [15].

The activated HSCs were seen mostly in the perisinusoidal spaces in case of active cirrhosis. High numbers of alpha-SMA-positive HSCs were also detected in the areas of inflammation and piecemeal necrosis, suggesting that HSCs were activated by compounds that were present mainly in the sites of inflammation. In inactive cirrhosis activated HSCs were distributed throughout the entire nodules even though a higher number was seen at the periphery of the regenerative liver plates. The lack of an evident zonal distribution of activated HSCs in advanced stages of cirrhosis even supported our previous observations of a lack of zonal compartmentalisation in the intra-nodular sinusoidal bed, together with loss of the ultrastructural and metabolic zonation of the hepatocytes, detected in CCl_4 cirrhosis experimentally induced in the rat [34].

In the present study, the morphometrical analysis has shown similar findings in both the HBV-C and HCV-C groups compared to the DL group: fibrosis and the volume occupied by portal tracts (arteries, veins and bile ducts) was increased, while that occupied by parenchyma (hepatocytes and sinusoids) was reduced. The HCV-PTR group was characterised by intermediate values of fibrosis, portal tract and parenchyma between normal and cirrhotic livers. The morphometrical similarities in the two cirrhotic groups, together with the fact that they did not differ in terms of HSCs activation and were of comparable staging, suggest that HSCs activation with fibrosis deposition and parenchymal reduction are not directly related to the aetiological factors of the liver disease, but represent stereotyped response to chronic insults similar to the wound healing process in other tissue including skin, lung and kidney [35,36]. HSC activation would be caused by paracrine stimulation of compounds, such as wound hormone TGF-Beta, PDGF, Il-1 and reactive oxygen species, produced by all neighbouring cell types including hepatocytes, Kupffer cells, platelets, sinusoidal endothelial cells and leukocytes [2,5,37–39].

In conclusion, alpha-SMA represented a reliable marker of HSC differentiation to myofibroblastic-like cells. Activation of HSC was correlated to the progression of hepatic fibrosis, but it did not seem to be related to the aetiological factors of the liver disease, probably because it represented a stereotyped response to chronic insults. Appearance of alpha-SMA-positive HSCs in recurrent HCV-hepatitis after liver transplantation confirmed that re-activation of HSCs coincided with the resumption of inflammatory stimuli and preceded the (re-)accumulation of scar substances. Although deeper studies are needed, the above findings suggest that alpha-SMA could be a useful marker to identify the earliest stages of hepatic fibrosis, as well as for monitoring the efficacy of the therapy.

Conflict of interest statement

None declared.

List of abbreviations

Alpha-SMAalpha smooth muscle actin; DL, donor livers; ECM, extra-cellular matrix; HAI, histology activity index; HBV-C, HBV cirrhosis; HCV-C, HCV cirrhosis; HCV-PTR, post-transplant HCV recurrent hepatitis; HSC, hepatic stellate cell.

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